Supplementary files for:

Two-stage electro-mechanical coupling of a Kv channel in voltage-dependent activation

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This PDF File Includes:

Supplementary Table 1

Supplementary Figures. 1 to 7

Supplementary Table 1. All primer sequences used in this study.

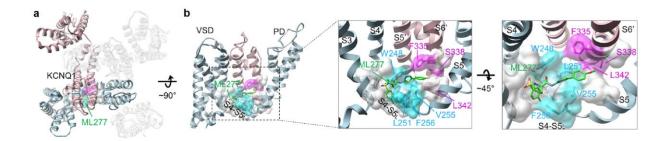
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pC: GAGGCCCAGGGCGCCGATGTAC pB: gatgaggccTTCgaagccgatgtacagg pC: cttcGAAggcctcatcttctcc pB: CTTCCTGGCCCTCATCTTCT pC: AGAAGATGAGGGCCAGGAAG pB: gaagatgaATcccaggaagccgatg	0207A	pC: AGGCCCAGGAAGGCGATGTACAG
pC: GAGGCCCAGGGCGCCGATGTAC pB: gatgaggccTTCgaagccgatgtacagg pC: cttcGAAggcctcatcttctcc pB: CTTCCTGGCCCTCATCTTCT pC: AGAAGATGAGGGCCAGGAAG pB: gaagatgaATcccaggaagccgatg	F270A	
pC: cttcGAAggcctcatcttctcc pB: CTTCCTGGCCCTCATCTTCT pC: AGAAGATGAGGGCCAGGAAG pB: gaagatgaATcccaggaagccgatg	T270A	pC: GAGGCCCAGGGCGCCGATGTAC
G272A pB: CTTCCTGGCCCTCATCTTCT pC: AGAAGATGAGGGCCAGGAAG pB: gaagatgaATcccaggaagccgatg	I 271E	pB: gatgaggccTTCgaagccgatgtacagg
pC: AGAAGATGAGGCCAGGAAG pB: gaagatgaATcccaggaagccgatg	1/4/11/2	pC: cttcGAAggcctcatcttctcc
pC: AGAAGATGAGGGCCAGGAAG pB: gaagatgaATcccaggaagccgatg	C272 A	pB: CTTCCTGGCCCTCATCTTCT
1.7.738	GZIZA	pC: AGAAGATGAGGGCCAGGAAG
DC: cotagg A Trestettetectestes	I 272E	pB: gaagatgaATcccaggaagccgatg
pc. cetgggArteatettetetegtae	L2/3F	pC: cctgggATtcatcttctcctcgtac

I274A	pB: TGGGCCTCGCCTTCTCGAGCTA
127 111	pC: TAGCTCGAGAAGGCGAGGCCCA
F275A	pB: GCCTCATCGCCTCGAGCTA
	pC: TAGCTCGAGGCGATGAGGC
F335A	pB: CTTCTCTGTCGCTGCCATCTCC
	pC: CTTCTCTGTCGCTGCCATCTCC
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F331A	pC: gctcgggCGCtgccctgaaggtgcag
A 352C	pB: GATCCGGGTTTTGCCTGAAGGTGCAG
A352C	pC: CTGCACCTTCAGGCAAAACCCGGATC
L353K	pB: ccttcTTggcaaaccccgagc
LJJJK	pC: gggtttgccAAgaaggtgcagcagaag
K354A	pB: ctgcacGGCcagggcaaaccccg
NJ34A	pC: ccctgGCCgtgcagcagaagcag
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V355W	pC: gggtttgccctgaagTGgcagc

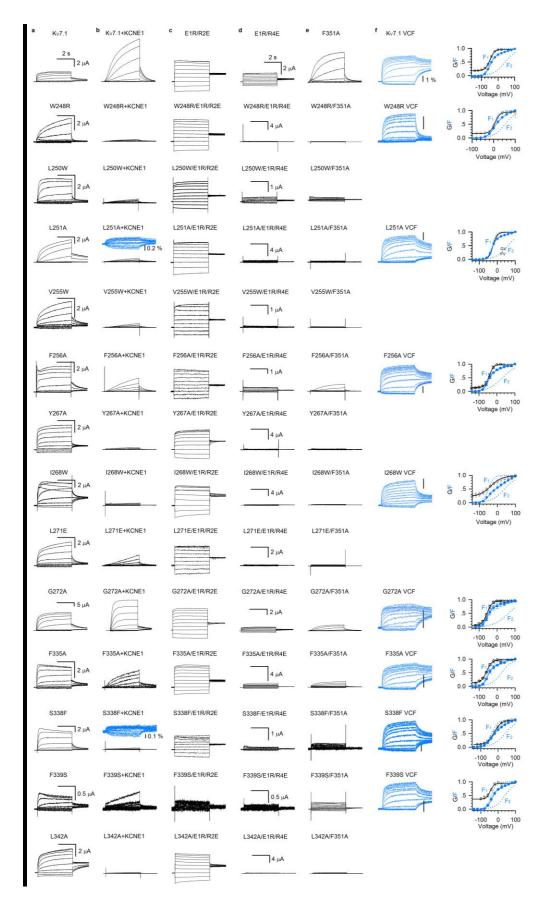
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Q356A	pC: CTGCTTCTGCGCCACCTTCAGGGCAAAC
03574	pB: GAAGGTGCAGGCGAAGCAGAGCAGA
Q357A	pC: TCTGCCTCTGCTTCGCCTGCACCTTC
K358A	pB: GTGCAGCAGGCGCAGAGGCAGAAGCAC
KSSOA	pC: GTGCTTCTGCCTCTGCGCCTGCTGCAC
02504	pB: GTGCAGCAGAAGGCGAGGCAGAAGCAC
Q359A	pC: GTGCTTCTGCCTCGCCTTCTGCTGCAC
D260A	pB: CAGCAGAAGCAGGCGCAGAAGCACTTC
R360A	pC: GAAGTGCTTCTGCGCCTGCTTCTGCTG
M238W	pB: gacgtgCagcCAcctcaggatctgcagg
W1238 VV	pC: gaggTGgctGcacgtcgaccgccag
E160R/	Use E160R/R237E as template
R237E/	M238W-pB: gacgtgCagcCAcTCcaggatctgcagg
M238W	M238W-pC: GAgTGgctGcacgtcgaccgccag
L239W	pB: cgacgtgCCAcatcctcaggatctgc
L239 VV	pC: ggatgTGGcacgtcgaccgccag
E160R/	Use E160R/R237E as template
R237E/	L239W-pB: gacgtgCCAcatcTCcaggatctgc
L239W	L239W-pC: GAgatgTGGcacgtcgaccgccag
D242W	pB: gcgCCAgacgtgtagcatcctc
D242 VV	pC: ctacacgtcTGGcgccagggaggcac
R243W	pB: ccctgCcAgtcgacgtgtagcatc
	pC: gtcgacTgGcagggaggcacctgg
I268A	pB: caggaaTccgGCgtacagggtggttatcag
1200A	pC: gtacGCcggAttcctgggcctcatcttc
L251F	pB: gagccGaAgagcctccaggtgcc
L/231F	pC: gaggeteTtCggeteegtggtette
F339L	pB: cgcGaaGaGggagatggcaaagacag
FJJJL	pC: catctccCtCttCgcgctcccagcggg

Supplementary Figures



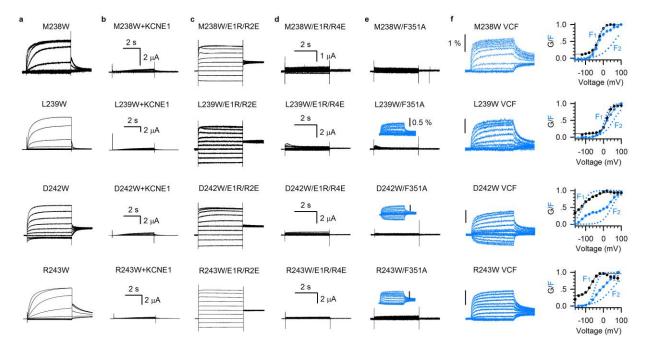
Supplementary Fig. 1, Molecular docking results of ML277 onto KCNQ1 channel.

(a) Residues interacting with ML277 based on molecular docking and experimental results. Top view of the binding mode of ML277 (green) at the homology model of $hK_V7.1$ (see Methods). Two neighboring subunits (light red and light blue) are highlighted. (b) Side view of the binding mode of ML277. Only two neighboring subunits (light red and light blue) are shown for clarity. Right panels, detailed structure of the ML277 (green) binding at the S4-S5L/pore interface. Interacting residues that consistent with mutagenesis results shown in cyan (S4-S5L) and magenta (pore).



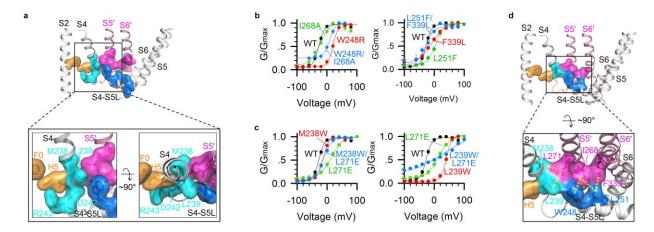
Supplementary Fig. 2. Dataset for functional validations of AO state E-M coupling residues identified by the ML277 screen.

(a-b) Currents of WT and mutant $K_V7.1$ with and without KCNE1. Currents are shown in the same scale. VCF experiments was performed on S338F+KCNE1 and L251A+KCNE1, indicating membrane expression and VSD function. (c-e) Currents of WT and mutant $K_V7.1$ on top of the IO background E1R/R2E, the AO background E1R/R4E and F351A. Currents are shown in the same scale. (f) VCF results of WT and eight mutations in $K_V7.1^*$. $n \ge 3$. Source data are provided as a Source Data file.



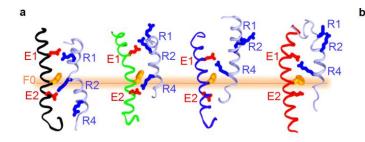
Supplementary Fig. 3. Dataset for functional validations of S4c residues critical for AO state E-M coupling.

(a-b) Currents of S4c mutations with and without KCNE1. Currents are shown in the same scale. (c-e) Currents of the S4c mutations on top of the IO background E1R/R2E, the AO background E1R/R4E and F351A. Currents are shown in the same scale. VCF results (three out of four) are shown in blue. (f) VCF results of the four mutations in S4c in $K_V7.1^*$. $n \ge 3$. Source data are provided as a Source Data file.

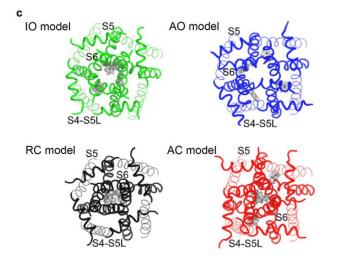


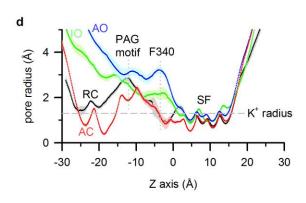
Supplementary Fig. 4. Double mutant cycle analysis.

(a) Mapping the key residues on to the Kv7.1 cryoEM structure, the VSD of which is at the activated state. Cyan, S4c residues (M238, L239, D242, and R243); Orange, F0 and H5 in the charge transfer center; Blue, S4-S5L residues; Pink, pore residues. Lower panels, detailed structure of the key residues. (b-c) Double mutant cycle analysis of the interactions at S4-S5L/pore interface: W248 and I268 ($\Delta\Delta G$ = 1.4 kcal/mol) and L251 and F339 ($\Delta\Delta G$ = 2.7 kcal/mol), as well as the S4c/S5 interface: M238 and L271 ($\Delta\Delta G$ = 1.7 kcal/mol) and L239 and L271 ($\Delta\Delta G$ = 4.4 kcal/mol). G–V relations from WT and mutations are fitted with Boltzmann equation. n \geq 3. (d) Mapping the interactions M238/L271 and L239/L271 at the S4c/S5 interface, and W248/I268, L251/I268, and L251/F339 at the S4-S5L/pore interface. Color code is the same as in a, and yellow dashed lines indicate the interactions.



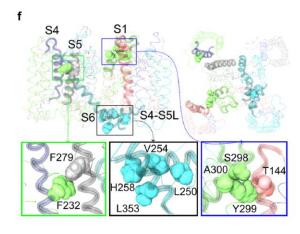
State-depend	dent salt	bridges i	in Kv7.1 i	nodels	
Salt bridge	Number of subunits (over 4)				
	RC	10	AO	AC	
E1/R1	3	0	0	0	
E2/R2	3	0	0	0	
E1/R2	1	4	0	0	
E2/R4	0	3	0	0	
E1/R4	0	0	4	4	

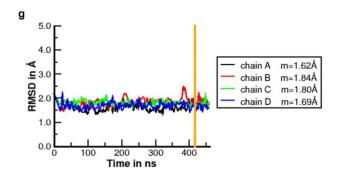


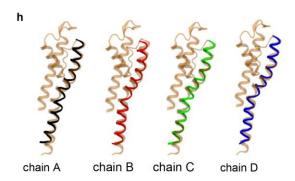


Neighbo	or residu	es in Kv	7.1 mode	ls
Residue	Number of subunits (over 4)			
pairs	RC	10	AO	AC
T144/S298	4	3	4	4
T144/Y299	4	4	4	4
T144/A300	4	3	4	4
F232/F279	3	4	4	4
L250/L353	4	4	4	0
V254/L353	4	4	4	4
H258/L353	4	4	4	4

е

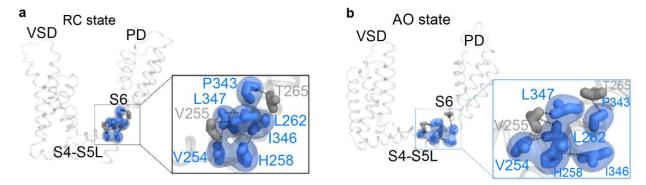






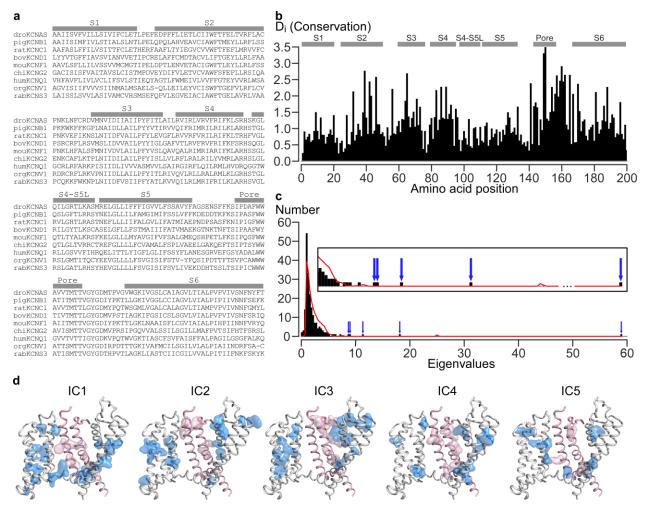
Supplementary Fig. 5. Structural validation of the MD trajectories obtained for Kv7.1 models.

(a) State-dependent K_V7.1 VSD salt-bridges¹⁻⁴. Representation of S2 (in black, green, blue and red) and S4 (in blue) segments in K_V7.1 RC, IO, AO and AC models, respectively. S4 gating charges are represented in blue sticks, while its S2 countercharges are represented in red sticks. The hydrophobic plug F167 (F0) is represented in orange sticks. (b) Summary table of statedependent K_V7.1 VSD salt-bridges in the subunits of K_V7.1 models. (c) Intracellular view of S4-S5L and PD segments in K_V7.1 IO (top, left), AO (top, right), RC (bottom, left) and AC (bottom, right) models. The gray spheres are sidechains of F340. (d) Average pore radii of the conduction pathways of K_V7.1 models. Standard deviations are represented by black, green, blue and red horizontal bars for the MD trajectories obtained from RC, IO, AO and AC models, respectively. (e) Summary table of state-dependent KCNQ1 neighbor residues pairs identified by cysteine scanning studies and previous double mutant cycle analysis in the subunits of KCNQ1 RC, IO, AO and AC models involving T144⁵, F232⁶, and L353⁷. In this analysis we calculated the distance between Cβ atoms of the residue pairs with a cut-off of 13 Å to determine if these residues are prone to interact when mutated into Cys. (f) Interacting pairs of KCNQ1 neighbor residues. In the upper panel, the side view (left) and the extracellular view (right) of the seven pairs of neighbor residues in the KCNQ1 IO model are shown. The transmembrane segments (in cartoon) bearing the residues of interest are highlighted in transparent colors. In the lower panel, the zoomed views of IO state specific interaction in IO model (left), RC state specific interactions in RC model (middle), and of AO state specific residue pairs in AO model (right) are displayed. (g-h) Comparison of the pore domain of K_V7.1 RC model with KCNQ1_{EM} structure. (g) The graph project the root-mean-square deviation (RMSD) of the pore domain segments of K_V7.1 RC model with respect to KCNQ1_{EM} structure throughout its MD simulation time (in ns). Average RMSD values are displayed for each subunit (labeled chain A, B, C, D). The orange vertical line represents the simulation time at which the pictures displayed in (h). (h) A cartoon representation of the structural alignment of the S6 segment of KCNQ1_{EM} structure (in transparent orange) with those of $K_V7.1$ (top) RC subunits, each captured at ~420 ns. Each subunit is represented in solid black, red, green and blue cartoon, respectively. Source data are provided in Source Data file.



Supplementary Fig. 6. Possible intra-subunit interactions at the classical E-M coupling region in RC and AO states.

(a-b) Representations of the IO and AO state models of a $K_V7.1$ subunit. Inset: enlarged structures of the boxed area. Blue, residues identified as important for E-M coupling in experiments (Fig. 1c-g); grey: V255 and T265, which were identified in MD simulations as important for E-M coupling, but for which mutations still show functional currents (Fig. 3c). Residue V255 was found to be important for the AO state E-M coupling (Fig. 3). Each pair of residues is represented by a black dashed line connecting their respective sidechains.



Supplementary Fig. 7. Statistical coupling analysis of domain-swapped Ky channels.

(a) Expanded sample MSA (from Figure 6a) used as input for analysis by SCA. MSA uses the nomenclature established by the HUGO Gene Nomenclature Committee (HGNC) for voltage-gated potassium channels⁸. K_V channels can adopt two types of architectures. In "domain-swapped" channels, the VSD from one subunit contacts the PD from the neighboring subunit. In "non-domain-swapped" channels, the VSD from one subunit contacts the PD from the same subunit. These distinct architectures likely give rise to different modes of E-M coupling. SCA was applied to the K_V channel superfamily previously⁹; however, prior studies utilized both domain-swapped and non-domain swapped K_V channels in the input data. In this MSA, only domain-swapped K_V channels were included. One member from each of $K_V1 - K_V9$ is shown. The final processed MSA includes 1421 sequences at 200 positions. (b) First order conservation by amino acid position within the MSA. D_i on the y-axis is calculated based on Kullback-Leibler divergence which measures deviation of the observed amino acid frequency in the MSA (Fig. 6a)

from the natural "background" amino acid frequency (see Methods). Bars indicate positions of the helical segments. (c) A histogram of eigenmodes tallied by their associated eigenvalues from SCA calculation (black). Red indicates the spectrum calculated by randomizing the input. Five significant eigenmodes can be seen by the black bars rising above the randomized red curve (blue arrows) at high eigenvalues. Inset shows enlarged scales. (d) Amino acid residues within independent component 1 through independent component 5 identified by SCA mapped onto the $K_V7.1$ cryoEM structure. Blue surfaces indicate all IC positions on one subunit, pink surfaces indicate the same IC positions on the neighboring subunit. Only the S5 and S6 helices are shown on the neighboring subunit for sake of clarity (pink helices). Accordingly, only the IC positions on the neighboring subunit on S5 and S6 are shown (pink surfaces). Using the IC submatrix and based on the strength of external correlation (see Methods, Figure 6c), IC2+IC3 are combined to form sector 1 (Fig. 6d), while IC1+IC4+IC5 are combined to form sector 2 (Fig. 6e). Source data for SCA are provided in Source Data file.

References

- 1. Hou, P. et al. Inactivation of KCNQ1 potassium channels reveals dynamic coupling between voltage sensing and pore opening. *Nat Commun* **8**, 1730 (2017).
- 2. Hou, P., Shi, J., White, K. M., Gao, Y. & Cui, J. ML277 specifically enhances the fully activated open state of KCNQ1 by modulating VSD-pore coupling. *Elife* **8** (2019).
- 3. Zaydman, M. A. et al. Domain-domain interactions determine the gating, permeation, pharmacology, and subunit modulation of the IKs ion channel. *Elife* **3**, e03606 (2014).
- 4. Wu, D., Pan, H., Delaloye, K. & Cui, J. KCNE1 remodels the voltage sensor of Kv7.1 to modulate channel function. *Biophys J* **99**, 3599-608 (2010).
- 5. Xu, Y. et al. Building KCNQ1/KCNE1 channel models and probing their interactions by molecular-dynamics simulations. *Biophys J* **105**, 2461-73 (2013).
- 6. Nakajo, K. & Kubo, Y. Steric hindrance between S4 and S5 of the KCNQ1/KCNE1 channel hampers pore opening. *Nat Commun* **5**, 4100 (2014).
- 7. Choveau, F. S. et al. KCNQ1 channels voltage dependence through a voltage-dependent binding of the S4-S5 linker to the pore domain. *J Biol Chem* **286**, 707-16 (2011).
- 8. Gutman, G. A. et al. International Union of Pharmacology. LIII. Nomenclature and molecular relationships of voltage-gated potassium channels. *Pharmacol Rev* **57**, 473-508 (2005).
- 9. Lee, S. Y., Banerjee, A. & MacKinnon, R. Two separate interfaces between the voltage sensor and pore are required for the function of voltage-dependent K(+) channels. *PLoS Biol* **7**, e47 (2009).